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Methanol exposure does not produce oxidatively damaged DNA in lung, liver or kidney of adult mice, rabbits or primates ☆

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ABSTRACT

In vitro and *in vivo* genotoxicity tests indicate methanol (MeOH) is not mutagenic, but carcinogenic potential has been claimed in one controversial long-term rodent cancer bioassay that has not been replicated. To determine whether MeOH could indirectly damage DNA via reactive oxygen species (ROS)-mediated mechanisms, we treated male CD-1 mice, New Zealand white rabbits and cynomolgus monkeys with MeOH (2.0 g/kg ip) and 6 h later assessed oxidative damage to DNA, measured as 8-oxo-2′-deoxyguanosine (8-oxodG) by HPLC with electrochemical detection. We found no MeOH-dependent increases in 8-oxodG in lung, liver or kidney of any species. Chronic treatment of CD-1 mice with MeOH (2.0 g/kg ip) daily for 15 days also did not increase 8-oxodG levels in these organs. These results were corroborated in DNA repair-deficient oxoguanine glycosylase 1 (*Ogg1*) knockout (KO) mice, which accumulated 8-oxodG in lung, kidney and liver with age, but exhibited no increase following MeOH, despite a 2-fold increase in renal 8-oxodG in *Ogg1* KO mice following treatment with a ROS-initiating positive control, the renal carcinogen potassium bromate ($KBrO_3$; 100 mg/kg ip). These observations suggest that MeOH exposure does not promote the accumulation of oxidatively damaged DNA in lung, kidney or liver, and that environmental exposure to MeOH is unlikely to initiate carcinogenesis in these organs by DNA oxidation.

Introduction

The carcinogenic potential of MeOH in humans remains unclear because there are no data available in human populations, and the limited data from long-term rodent cancer bioassays have reported conflicting results (NEDO, 1985a,b; Soffritti et al., 2002). In addition, due in part to the absence of an established animal model for MeOH carcinogenesis, there have been no studies demonstrating a molecular mechanism. At the Mitsubishi Kasei Institute for Toxicological and Environmental Sciences, Tokyo, Japan, the Japanese New Energy Development Organization (NEDO) conducted inhalation carcinogenicity studies in rats, mice and monkeys (NEDO, 1985a,b). No statistically significant methanol-related carcinogenic responses were seen in these studies; however, there was suggestive evidence of a potential increase in proliferative response in male rat lung. In contrast to the NEDO findings, treatment-related lymphohematoreticular neoplasms in Sprague–Dawley rats receiving methanol in drinking water were observed in studies conducted by the European Ramazzini Foundation for Oncology and Environmental Sciences (Soffritti et al., 2002). The validity of this study has been questioned based on the potential confounding influence of *Mycoplasma pulmonis* lung infection in the rats that were not specific pathogen-free, and the high background sensitivity to lymphoma in the study (EFSA, 2006; Cruzan, 2009; EFSA, 2009; Schoeb et al., 2009). The U.S. Environmental Protection Agency (EPA) has placed on hold its Integrated Risk Information System (IRIS) chemical carcinogenicity and toxicity assessment of MeOH based on recommendations of the U.S. National Toxicology Program (NTP) that further pathology reviews were needed to confirm the diagnoses of certain cancers reported in the Ramazzini Institute study.

One potential mechanism whereby MeOH could initiate carcinogenesis is via reactive oxygen species (ROS)-mediated oxidative damage to DNA. MeOH could initiate ROS formation directly via a free radical intermediate, or possibly indirectly through mechanisms like the activation and/or enhancement of ROS-producing NADPH oxidases, which has been reported for ethanol (Dong et al., 2010). A number of studies have reported evidence of free radical production during MeOH biotransformation (Skrzydlewska et al., 2000; Castro et al., 2002; Paula et al., 2003), most directly by the isolation of alpha-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN)-hydroxymethyl

Abbreviations: MeOH, methanol; ROS, reactive oxygen species; 8-oxodG, 8-oxo-2′-deoxyguanosine; 8-oxoG, 8-oxoguanine; OGG1, oxoguanine glycosylase 1; $KBRO_3$, potassium bromate; KO, knockout; POBN, alpha-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone; NEDO, New Energy Development Organization (of Japan).

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radical adducts in bile and urine from rats acutely intoxicated with methanol (Kadiiska and Mason, 2000).

8-Hydroxy-2′-deoxyguanosine (8-oxodG) is the most common ROS-initiated DNA lesion which, if unrepaired, causes post-replicative mispairing of 8-oxoguanine (8-oxoG) with adenine resulting in G:C to A:T transversion mutations (Klaunig and Kamendulis, 2004). A number of chemical carcinogens induce the formation of 8-oxodG specifically in their target organs (Kasai et al., 1987; Fiala et al., 1989), and animals models with decreased base excision repair of oxidatively damaged DNA have increased susceptibility to some cancers (Russo et al., 2004). In humans, the gene for 8-oxoguanine glycosylase 1 (OGG1), the primary enzyme for repair of 8-oxodG lesions, is located on chromosome 3p26.2, part of a region of the genome frequently deleted in several tumor types (Audebert et al., 2000), and there is a significant association between OGG1 polymorphisms (primarily S326C) and an increased cancer risk (review:(Goode et al., 2002)). Low OGG1 activity has been associated with an increased risk of human lung cancer (Paz-Elizur et al., 2003) and cigarette smoking status and hOGG1 genotype are interacting determinants of lung cancer risk (Chang et al., 2009).

The goal of the present study was to determine whether MeOH produces oxidatively damaged DNA, which could potentially lead to carcinogenesis. We evaluated this hypothesis in lung, kidney and liver from adult male mice, rabbits and primates (cynomolgous monkeys) for the following reasons: (a) humans and primates primarily use alcohol dehydrogenase (ADH) to metabolise MeOH to formaldehyde, whereas rodents use catalase (Liesivuori and Savolainen, 1991); rabbits may have enhanced hepatic ADH activity compared to rodents (Otani, 1978) and exhibit plasma MeOH and formic acid disposition profiles more similar than rodents to those in primates (Sweeting et al., 2010), as well as being more reflective than mice of human susceptibility to the ROS-initiating teratogen thalidomide (Parman et al., 1999); (b) the lung is an extrahepatic tissue with potential for high ROS formation and oxidatively damaged DNA (Parman et al., 1999), and a potential target organ for MeOH-initiated proliferative changes (NEDO, 1985b); and, (c) the kidney is an organ that does not metabolise MeOH, nor is it an expected target for MeOH carcinogenesis, but is a target organ for the renal carcinogen potassium bromate ($KBrO_3$), which as the ROS-initiating positive control specifically produces 8-oxodG (Kasai et al., 1987). To corroborate the above approach, as the steady state levels of oxidatively damaged DNA are a balance between the rate of formation and repair, we examined DNA repair-deficient knockout (KO) mice lacking oxoguanine glycosylase 1 (OGG1) to assess the possibility that 8-oxodG formation may be enhanced by MeOH but is obscured by rapid DNA repair via the base excision repair pathway. There was no evidence for MeOH-initiated oxidatively damaged DNA in any tissue from any species including *Ogg1* KO mice, despite enhanced 8-oxodG formation by the positive control $KBrO_3$, suggesting that MeOH would not initiate cancers in the organs investigated herein via DNA oxidation.

Materials and methods

Chemicals. HPLC grade MeOH was purchased from EMD Sereno Canada, Inc. (Mississauga, ON). Saline (0.9 %, sterile) was purchased from Baxter Corporation (Mississauga, ON). Nuclease P_1 from *Penicillium citrinum* (N8630), deferoxamine mesylate and 2′-deoxyguanosine (dG) were purchased from Sigma-Aldrich (St. Louis, MO). 8-hydroxy-2′-deoxyguanosine (8-oxodG) was obtained from Cayman Chemical Co. (Ann Arbor, MI). Calf intestine alkaline phosphatase (M0290S) was purchased from New England Biolabs Inc. (Pickering, ON). DNAzol was purchased from Molecular Research Center, Inc. (Cincinnati, OH). Chelex 100 chelating ion exchange resin was obtained from Bio-Rad Laboratories (Mississauga, ON). $KBRO_3$ was purchased from Alfa Aesar (Ward Hill, MA). All other reagents used were of analytical or HPLC grade.

Animals and diet. All animal protocols were approved by the institutional animal care committee in conformance with the guidelines established by the Canadian Council on Animal Care.

Mice. Male CD-1 mice were purchased from Charles River Laboratories (Saint-Constant, QC) and were between 9 and 13 weeks old at the time of study. *Ogg1* KO mice were generously provided by Dr. Tomas Lindahl (Imperial Cancer Research Fund, United Kingdom) through Dr. Christi A. Walter at the University of Texas Health Science Center at San Antonio, and were 6 months old at the time of study. Mice were housed in vented cages from Allentown, Inc. (Allentown, NJ) with ground corn cob bedding (Bed-O' Cobs Laboratory Animal Bedding, The Andersons Industrial Products Group, Maumee, OH). Animal rooms were climate- and light-controlled (20 °C, 50 % humidity, 14 h light–10 h dark cycle). Mice were fed rodent chow (Harlan Labs: 2018, Harlan Teklad, Montreal, QC) and provided with water *ad libitum*.

Rabbits. Male New Zealand white (NZW) rabbits were purchased from Charles River Laboratories. At the time of experiments, rabbits were 5 months old with a weight range of 3.25–3.75 kg. Rabbits were housed in plastic cages (Allentown, Inc.) in rooms maintained at 20 °C and 60% humidity, with an automated 12 h light–dark cycle. Rabbits were fed a diet of standard high-fiber rabbit chow (Lab Diet: 5236 Hi-Fiber, PMI Nutrition International LLC, Brentwood, MO), and provided with water *ad libitum*.

Primates. Studies were conducted with male cynomolgus monkeys (*Macaca fascicularis*) at Charles River Laboratories (Sparks, NV). At the time of experiments, monkeys were between the ages of 3.4–5.7 years, with a weight range of 2.8–4.8 kg. Monkeys were acclimatized to individual stainless steel cages 2 weeks prior to commencement of the study in rooms maintained between 18 and 29 °C, with an automated 12 h light-dark cycle. Monkeys were fed a certified primate chow diet (# 5048) from Purina Mills (St. Louis, MO) supplemented with fruit or vegetables 2–3 times weekly, and provided with water *ad libitum*.

Dosing and tissue collection. We studied the effect of a limit dose of 2.0 g/kg bw MeOH based on guidelines established for the comet assay developed in accordance with the *in vivo* genetic toxicology guidelines of the Organization for Economic Co-operation and Development (OECD) (Tice et al., 2000). Mice were given a single dose or daily doses for 15 consecutive days of 2.0 g/kg bw MeOH (20% [w/v] in sterile saline) or saline vehicle (controls) between 8:30 a.m. and 9:30 a.m. $KBRO_3$ was dissolved with sterilized 0.9% saline, and $KBRO_3$ or its vehicle were given in a fixed volume of 0.1 ml/10 g body weight at a dose 100 mg/kg bw. Drugs were administered via intraperitoneal (ip) injection using a 26 gauge (G) 3/8 needle. Mice were sacrificed by cervical dislocation. Organs were rapidly removed, flash frozen in liquid nitrogen, and stored at −80°C until time of analysis. Rabbits were administered a single dose (2 g/kg bw) of MeOH (20% [w/v] in sterile saline) or its saline vehicle (control) by ip injection using a 23 G needle. Rabbits were sacrificed by CO_2 asphyxiation 6 h post-dose and organs rapidly dissected, and flash frozen in liquid nitrogen. Tissues were stored at −80°C until time of analysis. Primates were lightly sedated with ketamine (~5–10 mg/kg) immediately prior to treatment and then administered MeOH (2 g/kg bw; 20% [w/v] in sterile saline) or its saline vehicle by ip injection using a 22 G needle. Primates were sacrificed 6 h post-dose by pentobarbital overdose, followed by whole body perfusion with phosphate buffered saline. Organs were rapidly dissected, flash frozen in liquid nitrogen, and stored at −80°C until use.

Analytical methods. DNA was isolated using DNAzol, a novel guanidine-detergent lysing solution that hydrolyzes RNA and allows for the selective precipitation of DNA from cell lysates (Chomczynski et al., 1997). All buffers and aqueous solutions were treated with Chelex 100 to reduce transition metal contamination (Buettner, 1988). Immediately before use DNAzol was supplemented with 1 mM deferoxamine to chelate transition metals, and 1 mL DNAzol/50 mg tissue sample was homogenized on ice using a motorized 5 ml glass-Teflon Potter-Elvehjem homogenizer. The samples were stored for 10 min at room temperature and then sedimented at 2400g for 10 min. DNA was precipitated from the supernatant using 0.5 mL ethanol per mL of supernatant. The DNA was sedimented at 2400g and washed three times with 75% aqueous ethanol prior to dissolution in 20 mM Na-acetate buffer pH 5.2 containing 0.1 mM deferoxamine. Diluted DNA samples were digested for 1 h at 37 °C with 5 U of nuclease P1 prepared in 20 mM Na-acetate buffer. The pH was adjusted to 8.5 with 1 M Tris-HCl buffer and the DNA hydrolyzed to nucleosides by incubation with 6 U of calf intestine alkaline phosphatase for 1 h at 37 °C. The suspension was transferred to Amicon YM-10 spin columns and centrifuged at 14,000g for 30 min, filter-sterilized and injected for analysis by high-performance liquid chromatography (HPLC). The HPLC system consisted of a 0.2 μm mobile phase filter, an isocratic HPLC pump (200 series, Perkin Elmer, Boston, MA) set at 1.0 mL/min, a pulse dampener (LP-21 LO-Pulse; Scientific Instruments, Inc., State College, PA), a chilled (4 °C) autosampler (200 series, Perkin Elmer, Boston, MA) with 200 μL loop and a C_{18} guard column (Supelcosil LC 18-T, 20 mm, 5 μm; Sigma-Aldrich, St. Louis, MO) connected to a C_{18} reverse-phase column (Supelcosil LC 18-T, 250×4.6 mm, 5 μm; Sigma-Aldrich, St. Louis, MO). The 8-oxo-dG was detected with an electrochemical detector (Coulochem5200A, ESA, Chelmsford, MA) with an analytical cell (5011, ESA, 5 nA; screen electrode, 100 mV; analytical electrode, 400 mV). A UV detector (200 series, Perkin Elmer, MA) at 280 nm was used to detect dG and data were recorded using a NCI 902 dual detector interface (Perkin Elmer, Boston, MA). The HPLC buffer consisted of 7.5% methanol, Milli-Q grade water and 50 mM sodium acetate buffer, pH 5.3, filtered through a 0.2 μm filter (Millipore, Bedford, MA). 8-OxodG and dG were quantified from standard curves prepared from authentic standards for which the concentrations were verified by UV spectrometry using a molar extinction coefficient of 12,300 M^{-1} cm^{-1} at 247 nm for both dG and 8-oxodG (Williamson et al., 2003). Genotyping of Ogg1 mice was performed as previously described (Wong et al., 2008).

Data analysis. Statistical analysis was performed using GraphPad Instat Version 3.05 (GraphPad Software, Inc., San Diego, CA). Experiments comparing two groups were analyzed by unpaired Student's *t* tests and multiple comparisons were analyzed by one-way ANOVA followed by a Tukey post-test. The level of significance was set at $P<0.05$.

Results

No increase in oxidatively damaged DNA was observed in lung, liver or kidney at 6 h following exposure to MeOH (2.0 g/kg bw ip) in mice, rabbits or primates (Figs. 1–3). Similarly, no increase in DNA oxidation was observed in lung, liver, or kidney 24 h following exposure to a single dose of MeOH (2.0 g/kg bw ip), or following 15 consecutive daily doses of 2.0 g/kg bw ip MeOH in CD-1 mice (Figs. 1–4).

No increase in oxidatively damaged DNA was observed in lung, liver or kidney of wild-type *Ogg1*-normal mice (+/+) or homozygous DNA repair-deficient *Ogg1* KO mice (−/−) following a single MeOH (2.0 g/kg i.p. bw) dose at 6 or 24 h post dose (Fig. 5). In contrast, oxidatively damaged DNA accumulated in lung, liver and kidney of *Ogg1* (−/−) DNA repair–deficient KO mice compared to wild-type (+/+) repair-normal controls. Comparison of *Ogg1* (+/+) to *Ogg1* (−/−) samples revealed average increases in 8-oxodG levels in *Ogg1* (−/−) mice of 1.7-, 2.0- and 2.1-fold for lung, liver and kidney, respectively (Fig. 5). Exposure to the ROS-initiating renal carcinogen $KBRO_3$ (100 mg/kg ip) produced a 2-fold increase in the level of 8-oxodG in renal DNA of *Ogg1* (−/−) mice (Fig. 6).

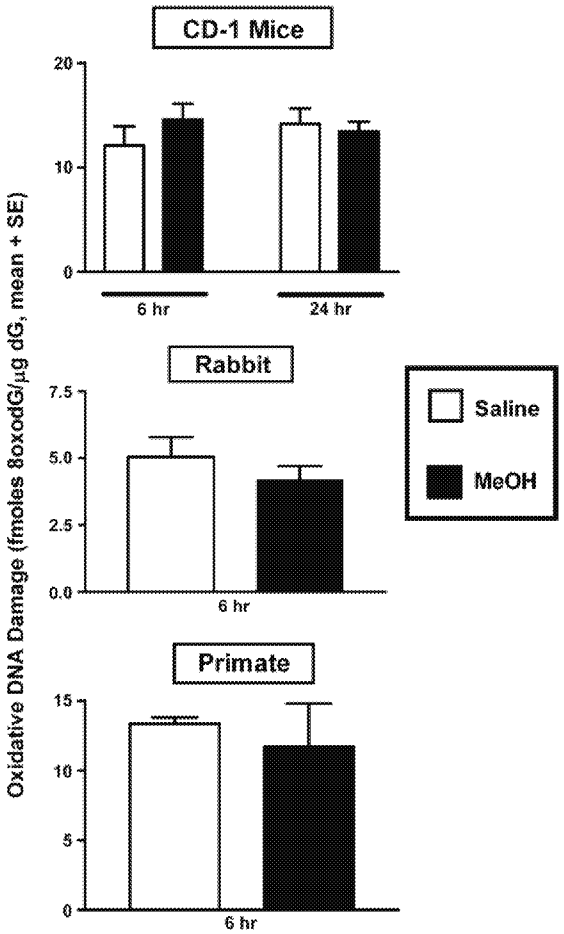

Fig. 1. Oxidatively damaged DNA is not increased in lung from male CD-1 mice, New Zealand white rabbits or cynomolgus monkeys following acute exposure to methanol (2.0 g/kg ip). Animals were treated intraperitoneally with a single dose of 2.0 g/kg bw MeOH (20% [w/v] in sterile saline) or saline vehicle (controls) and sacrificed at 6 or 24 h post-injection. Genomic DNA was isolated and analyzed for oxidatively damaged DNA damage reflected by the formation of 8-hydroxy-2'-deoxyguanosine (8-oxodG). Values are mean + SE; $N=4$ for mice and $N=3$ for rabbit and monkeys, respectively.

Discussion

The carcinogenic potential of MeOH in humans remains uncertain due to conflicting data in long-term rodent cancer bioassays (NEDO, 1985a,b; Soffritti et al., 2002) and a lack of understanding of potential molecular mechanism(s) that could account for MeOH-initiated carcinogenesis.

Oxidatively damaged DNA has been implicated as a causative factor in carcinogenesis for decades (Ames, 1989). 8-OxoG is the most prevalent promutagenic oxidation product of guanine, yielding G-to-T transversion mutations that may activate oncogenes or inactivate tumour suppressor genes known to be linked to the development of cancers (Hsu et al., 2004; Klaunig and Kamendulis, 2004). Studies in genetically modified mice with deficiencies in DNA glycosylases that protect against G-to-T transversions provide strong evidence of a causal role for oxidatively damaged DNA in tumourigenesis (Russo et al., 2004; Xie et al., 2004; Kinoshita et al., 2007). Dimethylarsinic acid strongly increases 8-oxodG levels and carcinogenicity in lungs of

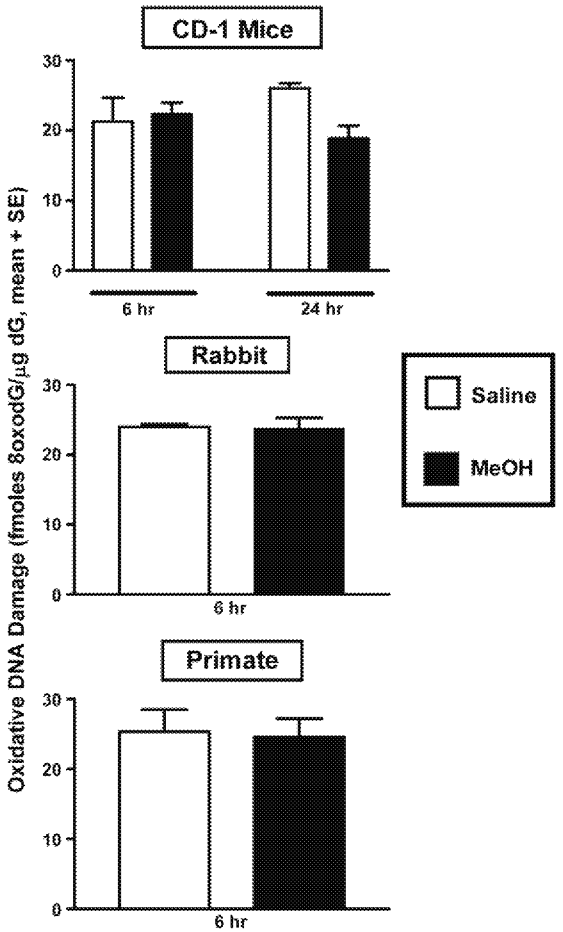

Fig. 2. Oxidatively damaged DNA is not increased in liver from male CD-1 mice, New Zealand white rabbits or cynomolgus monkeys following acute exposure to methanol (2.0 g/kg ip). Animals were treated with methanol and tested for oxidatively damaged DNA as described in Fig. 1. Values are mean + SE; $N = 4$ for mice and $N = 3$ for rabbit and monkeys, respectively.

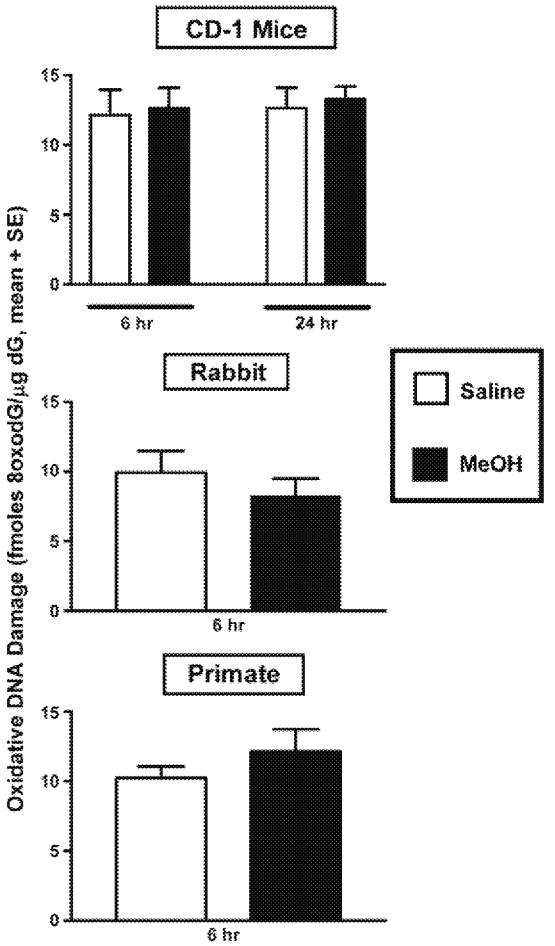

Fig. 3. Oxidatively damaged DNA is not increased in kidney from male CD-1 mice, New Zealand white rabbits or cynomolgus monkeys following acute exposure to methanol (2.0 g/kg ip). Animals were treated with methanol and tested for oxidatively damaged DNA as described in Fig. 1. Values are mean + SE; $N = 4$ for mice and $N = 3$ for rabbit and monkeys, respectively.

Ogg1 KO mice (Kinoshita et al., 2007). In double mutant mice deficient for *Ogg1* and *Myh*, 8-oxodG accumulates in lung and small intestine, and these organs have multifold increases in cancer incidence with a high frequency of occurrence of G-to-T transversion mutations that activate the K-ras oncogene in the lung cancers (Xie et al., 2004). Deficiencies in repair of 8-oxoG have also been suggested to be risk factors for the development of human lung cancer (Paz-Elizur et al., 2003; Mambo et al., 2005). Accordingly, the study herein investigated the hypothesis that free radical-initiated, ROS-mediated oxidative damage to DNA could be a potential mechanism whereby MeOH might initiate pulmonary carcinogenesis.

No increase in oxidatively damaged DNA was observed in lung, liver or kidney 6 h following the limit dose in any species. Further analysis in CD-1 mice revealed no effect of MeOH on oxidatively damaged DNA in lung, liver or kidney 24 h following a single MeOH 2.0 g/kg ip dose, or following 15 consecutive daily doses of 2.0 g/kg ip.

To rule out the possibility that the failure of MeOH to cause DNA oxidation was due to masking by rapid repair of initiated lesions, we performed further studies in DNA repair-deficient *Ogg1* KO mice. Our results in CD-1 mice were corroborated in both wild-type and *Ogg1* KO mice, in which where there was no effect of MeOH (2.0 g/kg bw) on the accumulation of oxidatively damaged DNA at either 6 or 24 h post dose. These observations are in contrast to dimethylarsinic acid, a known human pulmonary carcinogen, where a 1.7-fold increase in 8-oxodG levels was reported in *Ogg1* KO mice (Kinoshita et al., 2007) and the renal carcinogen, $KBRO_3$ which doubled the levels of 8-oxodG in renal DNA of *Ogg1* KO mice in this study. Importantly, the increases in basal levels of 8-oxodG in the lung, liver and kidney of *Ogg1* KO mice compared to wild-type controls were consistent with the accumulation of oxidatively damaged DNA occurring through normal cellular metabolism that would be expected in repair-deficient *Ogg1* mice (Kinoshita et al., 2007).

Despite the negative findings herein for enhanced oxidatively damaged DNA using a limit dose of 2.0 g/kg bw MeOH, a number of studies have reported the formation of free radicals during MeOH biotransformation (Skrzydlewska et al., 2000; Castro et al., 2002; Paula et al., 2003). The most direct *in vivo* evidence was the isolation of alpha-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (POBN)-hydroxymethyl radical adducts in bile and urine from rats acutely intoxicated with MeOH (4.5 or 7.5 g/kg ip) (Kadiiska and Mason, 2000). This dose was associated with liver damage determined by elevations in the liver enzymes alanine aminotransferase and sorbitol dehydrogenase 2 h post dose. In contrast to studies with ethanol (Reinke et al., 1987; Knecht et al., 1990), there was no evidence of lipid-derived radical generation in this study with high levels of MeOH exposure (Kadiiska and Mason, 2000). The mechanism of formation of the hydroxymethyl radical is not clear, although radical formation was enhanced by the ADH inhibitor 4-methylpyrazole, but not by the catalase inhibitor 3-aminotriazole, possibly by increasing the availability of MeOH to free radical generating pathways. However, 4-methylpyrazole also inhibits catalase-dependent alcohol metabolism by reducing the supply of H_2O_2 via inhibition of fatty acyl CoA oxidase (Bradford et al., 1993),

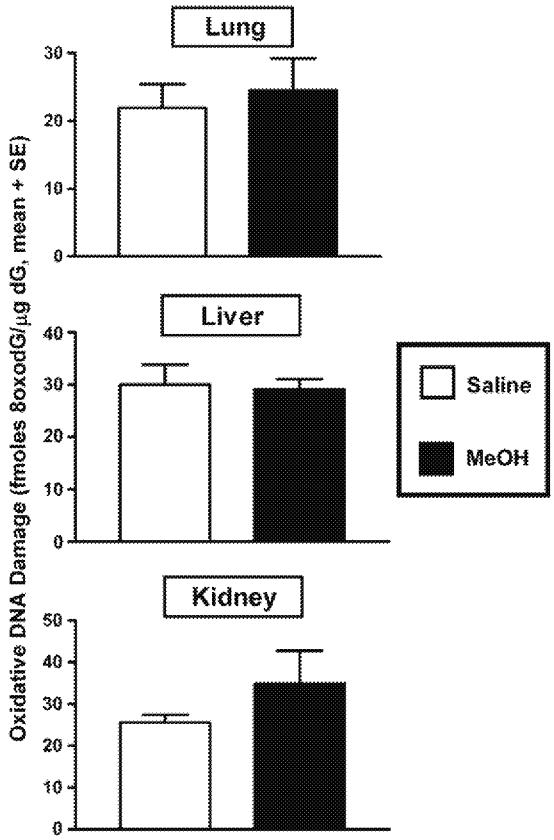

Fig. 4. Oxidatively damaged DNA is not increased in lung, liver and kidney from male CD-1 mice following chronic exposure to methanol (2.0 g/kg ip). Mice were given daily ip injections of 2.0 g/kg bw MeOH (20% [w/v] in sterile saline) or saline vehicle (controls) for 15 days and sacrificed at 24 h after the final injection. Genomic DNA was isolated and analyzed for oxidatively damaged DNA reflected by the formation of 8-hydroxy-2′-deoxyguanosine (8-oxodG). Values are mean + SE; $N = 5$.

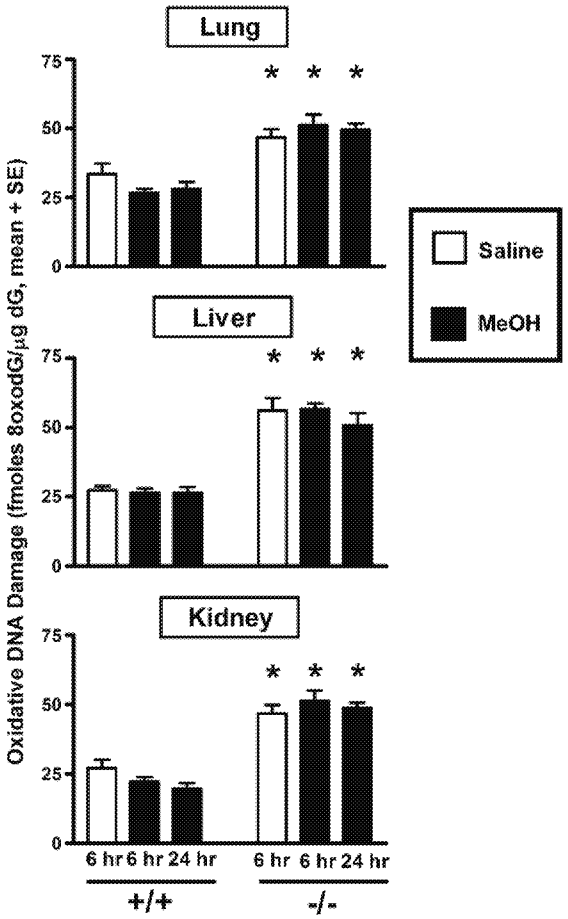

Fig. 5. Oxidative DNA damage accumulates with age in lung, liver, and kidney of *Ogg1* DNA repair-deficient knockout (KO) mice, but exposure to methanol does not increase 8-oxodG levels in lung, liver and kidney of *Ogg1* wild-type (+/+) or *Ogg1* KO (−/−) mice. Mice were given a single dose of 2.0 g/kg bw MeOH (20% [w/v] in sterile saline) or saline vehicle (controls) and sacrificed at 6 or 24 h post-injection. Genomic DNA was isolated and analyzed for oxidatively damaged DNA reflected by the formation of 8-hydroxy-2′-deoxyguanosine (8-oxodG). Values are mean + SE; N = 4. * denotes significant difference in Ogg1 (−/−) samples compared to the respective group of Ogg1 (+/+) mice $P < 0.05$.

and there is controversy in the interpretation of 3-aminotriazole inhibition experiments (Kato et al., 1987; Thurman and Handler, 1989).

An additional potential mechanism of MeOH-initiated carcinogenesis not investigated herein is the reaction of the primary metabolite of MeOH, formaldehyde, with DNA. Formaldehyde is a known carcinogen that causes nasopharyngeal cancer in humans and sqamous cell carcinomas of the respiratory epithelium of rats and mice (Kerns et al., 1983; IARC, 2006). Formaldehyde has not been detected in tissues or body fluids following toxic methanol exposures (Tephly, 1991), and is rapidly cleared from the bloodstream following intravenous infusion with a half-life between 1 and 1.5 min that is associated with a corresponding increase in blood formate levels (Malorny et al., 1965; McMartin et al., 1979). In a preliminary report, exposure of Sprague–Dawley rats with methanol (20,000 ppm) or formaldehyde (1,500 ppm) in the drinking water for 7 days failed to increase the basal levels of the major formaldehyde-DNA adduct N^6-hydroxymethyldeoxyadenosine (N^6-HOMe-dAdo) in leukocyte or hepatocyte DNA (Wang et al., 2008). Formaldehyde is an essential metabolic intermediate in all living cells and is endogenously produced from amino acid metabolism with the endogenous concentration of formaldehyde in the blood of human subjects estimated to be about 0.1 mM (IARC, 2006). A recent inhalation study using radiolabeled formaldehyde exposures at a dose of 10 ppm indicated that exogenous formaldehyde DNA adducts were observed only in nasal DNA, and that the exogenous adduct levels did not exceed the levels of endogenous formaldehyde DNA adducts, suggesting that mechanism(s) in addition to DNA adduct formation are involved in formaldehyde-dependent nasalpharyngeal cancer (Lu et al., 2010).

Acetaldehyde, the primary metabolite of ethanol, has been linked to the formation of esophageal cancers in patients with inherited deficiency in the enzyme aldehyde dehydrogenase 2 (ALDH2) (Brooks et al., 2009). The ALDH2-deficiency is not likely to strongly impact

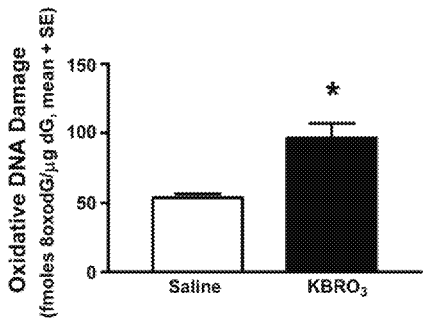

Fig. 6. Oxidatively damaged DNA is increased in renal DNA of *Ogg1* KO mice following exposure to the ROS-initiating renal carcinogen $KBRO_3$ (100 mg/kg ip). Mice were given a single dose of 2.0 g/kg bw MeOH (20% [w/v] in sterile saline) or saline vehicle (controls) and sacrificed 24 h post-injection. Genomic DNA was isolated and analyzed for oxidatively damaged DNA reflected by the formation of 8-hydroxy-2′-deoxyguanosine (8-oxodG). Values are mean + SE; $N = 4$. * denotes significant difference in $KBRO_3$ exposed group compared to the saline group $P < 0.05$.

formaldehyde disposition as early biochemical studies indicated the Km for formaldehyde of the cytosolic GSH-dependent formaldehyde dehydrogenase is an order of magnitude lower than that of mitochondrial aldehyde dehydrogenases (Cinti et al., 1976; Dicker and Cederbaum, 1984; Dicker and Cederbaum, 1986).

Despite numerous reports of MeOH-initiated free radical formation following high-dose exposures in rodents, the significance of these findings to low-dose environmental exposures (Kavet and Nauss, 1990) in humans remains questionable. The results presented herein suggest that even at limit dose exposure levels, MeOH does not produce oxidatively damaged DNA in lung, liver or kidney, which is consistent with other studies that found no genotoxic effects with MeOH (IPCS, 1997).

In summary, in vivo exposure to methanol at a dose of 2.0 g/kg bw did not increase the levels of the mutagenic DNA lesion 8-oxodG in lung, liver or kidney in any species. This lack of effect for MeOH does not appear to be due to masking by repair of the lesion, as no increase was observed in 8-oxoG repair-deficient *Ogg1* KO mice. In contrast, exposure to the renal carcinogen $KBRO_3$ increased renal 8-oxodG levels in *Ogg1* KO mice, and 8-oxodG accumulated with age in lung, liver and kidney of *Ogg1* KO mice. Taken together these observations suggest that it is unlikely that exposure to MeOH would initiate carcinogenicity in these organs via formation of mutagenic oxidatively damaged DNA.

Conflict of interest statement

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References

Ames, B.N., 1989. Mutagenesis and carcinogenesis: endogenous and exogenous factors. Environ. Mol. Mutagen. 14 (Suppl 16), 66–77.

Audebert, M., Chevillard, S., Levalois, C., Gyapay, G., Vieillefond, A., Klijanienko, J., Vielh, P., El Naggar, A.K., Oudard, S., Boiteux, S., Radicella, J.P., 2000. Alterations of the DNA repair gene OGG1 in human clear cell carcinomas of the kidney. Cancer Res. 60, 4740–4744.

Bradford, B.U., Forman, D.T., Thurman, R.G., 1993. 4-Methylpyrazole inhibits fatty acyl coenzyme synthetase and diminishes catalase-dependent alcohol metabolism: has the contribution of alcohol dehydrogenase to alcohol metabolism been previously overestimated? Mol. Pharmacol. 43, 115–119.

Brooks, P.J., Enoch, M.A., Goldman, D., Li, T.K., Yokoyama, A., 2009. The alcohol flushing response: an unrecognized risk factor for esophageal cancer from alcohol consumption. PLoS Med. 6, e50.

Buettner, G.R., 1988. In the absence of catalytic metals ascorbate does not autoxidize at pH 7: ascorbate as a test for catalytic metals. J. Biochem. Biophys. Meth. 16, 27–40.

Castro, G.D., Costantini, M.H., Delgado de Layno, A.M., Castro, J.A., 2002. Rat liver microsomal and nuclear activation of methanol to hydroxymethyl free radicals. Toxicol. Lett. 129, 227–236.

Chang, C.H., Hsiao, C.F., Chang, G.C., Tsai, Y.H., Chen, Y.M., Huang, M.S., Su, W.C., Hsieh, W.S., Yang, P.C., Chen, C.J., Hsiung, C.A., 2009. Interactive effect of cigarette smoking with human 8-oxoguanine DNA N-glycosylase 1 (hOGG1) polymorphisms on the risk of lung cancer: a case-control study in Taiwan. Am. J. Epidemiol. 170, 695–702.

Chomczynski, P., Mackey, K., Drews, R., Wilfinger, W., 1997. DNAzol: a reagent for the rapid isolation of genomic DNA. Biotechniques 22, 550–553.

Cinti, D.L., Keyes, S.R., Lemelin, M.A., Denk, H., Schenkman, J.B., 1976. Biochemical properties of rat liver mitochondrial aldehyde dehydrogenase with respect to oxidation of formaldehyde. J. Biol. Chem. 251, 1571–1577.

Cruzan, G., 2009. Assessment of the cancer potential of methanol. Crit. Rev. Toxicol. 39, 347–363.

Dicker, E., Cederbaum, A.I., 1984. Effect of acetaldehyde and cyanamide on the metabolism of formaldehyde by hepatocytes, mitochondria, and soluble supernatant from rat liver. Arch. Biochem. Biophys. 232, 179–188.

Dicker, E., Cederbaum, A.I., 1986. Inhibition of the low-Km mitochondrial aldehyde dehydrogenase by diethyl maleate and phorone in vivo and in vitro. Implications for formaldehyde metabolism. Biochem. J. 240, 821–827.

Dong, J., Sulik, K.K., Chen, S.Y., 2010. The role of NOX enzymes in ethanol-induced oxidative stress and apoptosis in mouse embryos. Toxicol. Lett. 193, 94–100.

EFSA, 2006. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to a new long-term carcinogenicity study on aspartame. Eur. Food Saf. Authority J. 356, 1–44.

EFSA, 2009. Updated scientific opinion of the panel on food additives and nutrient sources added to food on a request from the European commission related to the 2nd ERF carcinogenicity study on aspartame, taking into consideration study data submitted by the Ramazzini foundation in February 2009. Eur. Food Saf. Authority J. 1015, 1–18.

Fiala, E.S., Conaway, C.C., Mathis, J.E., 1989. Oxidative DNA and RNA damage in the livers of Sprague–Dawley rats treated with the hepatocarcinogen 2-nitropropane. Cancer Res. 49, 5518–5522.

Goode, E.L., Ulrich, C.M., Potter, J.D., 2002. Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol. Biomark. Prev. 11, 1513–1530.

Hsu, G.W., Ober, M., Carell, T., Beese, L.S., 2004. Error-prone replication of oxidatively damaged DNA by a high-fidelity DNA polymerase. Nature 431, 217–221.

IARC, 2006. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol.. IARC Monogr. Eval. Carcinog. Risks Hum. 88, 1–287.

IPCS, 1997. Methanol: Environmental health criteria, 196. World Health Organization, Geneva.

Kadiiska, M.B., Mason, R.P., 2000. Acute methanol intoxication generates free radicals in rats: an ESR spin trapping investigation. Free Radic. Biol. Med. 28, 1106–1114.

Kasai, H., Nishimura, S., Kurokawa, Y., Hayashi, Y., 1987. Oral administration of the renal carcinogen, potassium bromate, specifically produces 8-hydroxydeoxyguanosine in rat target organ DNA. Carcinogenesis 8, 1959–1961.

Kato, S., Alderman, J., Lieber, C.S., 1987. Ethanol metabolism in alcohol dehydrogenase deficient deermice is mediated by the microsomal ethanol oxidizing system, not by catalase. Alcohol Alcohol. Suppl. 1, 231–234.

Kavet, R., Nauss, K.M., 1990. The toxicity of inhaled methanol vapors. Crit. Rev. Toxicol. 21, 21–50.

Kerns, W.D., Pavkov, K.L., Donofrio, D.J., Gralla, E.J., Swenberg, J.A., 1983. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res. 43, 4382–4392.

Kinoshita, A., Wanibuchi, H., Morimura, K., Wei, M., Nakae, D., Arai, T., Minowa, O., Noda, T., Nishimura, S., Fukushima, S., 2007. Carcinogenicity of dimethylarsinic acid in Ogg1-deficient mice. Cancer Sci. 98, 803–814.

Klaunig, J.E., Kamendulis, L.M., 2004. The role of oxidative stress in carcinogenesis. Annu. Rev. Pharmacol. Toxicol. 44, 239–267.

Knecht, K.T., Bradford, B.U., Mason, R.P., Thurman, R.G., 1990. In vivo formation of a free radical metabolite of ethanol. Mol. Pharmacol. 38, 26–30.

Liesivuori, J., Savolainen, H., 1991. Methanol and formic acid toxicity: biochemical mechanisms. Pharmacol. Toxicol. 69, 157–163.

Lu, K., Collins, L.B., Ru, H., Bermudez, E., Swenberg, J.A., 2010. Distribution of DNA adducts caused by inhaled formaldehyde is consistent with induction of nasal carcinoma but not leukemia. Toxicol. Sci. 116, 441–451.

Malorny, G., Rietbrock, N., Schneider, M., 1965. The oxidation of formaldehyde to formic acid in the blood, a contribution to the metabolism of formaldehyde. Naunyn-Schmiedebergs Arch. Exp. Pathol. Pharmakol. 250, 419–436.

Mambo, E., Chatterjee, A., de Souza-Pinto, N.C., Mayard, S., Hogue, B.A., Hoque, M.O., Dizdaroglu, M., Bohr, V.A., Sidransky, D., 2005. Oxidized guanine lesions and hOgg1 activity in lung cancer. Oncogene 24, 4496–4508.

McMartin, K.E., Martin-Amat, G., Noker, P.E., Tephly, T.R., 1979. Lack of a role for formaldehyde in methanol poisoning in the monkey. Biochem. Pharmacol. 28, 645–649.

NEDO, 1985a. 18-month inhalation carcinogenicity study of methanol in B6C3F1 mice (test no.: 4A-223), Report dated March 30, 1985, 9 volumes. Mitsubishi Kasei Institute for Toxicological and Environmental Sciences, Tokyo. Available at www.methanol.org.

NEDO, 1985b. 24-month inhalation carcinogenicity study on methanol in Fischer rats (test no.: 5A-268), Report dated September 30, 1985, 10 volumes. Mitsubishi Kasei Institute for Toxicological and Environmental Sciences, Tokyo. Available at www.methanol.org.

Otani, M., 1978. Alcohol dehydrogenase activity in animal tissues. Nichidai Igaku Zasshi 37, 341–350.

Parman, T., Wiley, M.J., Wells, P.G., 1999. Free radical-mediated oxidative DNA damage in the mechanism of thalidomide teratogenicity. Nat. Med. 5, 582–585.

Paula, E.M., Mathangi, D.C., Namasivayam, A., 2003. Free radical changes in methanol toxicity. Indian J. Physiol. Pharmacol. 47, 207–211.

Paz-Elizur, T., Krupsky, M., Blumenstein, S., Elinger, D., Schechtman, E., Livneh, Z., 2003. DNA repair activity for oxidative damage and risk of lung cancer. J. Natl Cancer Inst. 95, 1312–1319.

Reinke, L.A., Lai, E.K., DuBose, C.M., McCay, P.B., 1987. Reactive free radical generation in vivo in heart and liver of ethanol-fed rats: correlation with radical formation in vitro. Proc. Natl Acad. Sci. USA 84, 9223–9227.

Russo, M.T., De Luca, G., Degan, P., Parlanti, E., Dogliotti, E., Barnes, D.E., Lindahl, T., Yang, H., Miller, J.H., Bignami, M., 2004. Accumulation of the oxidative base lesion 8-

hydroxyguanine in DNA of tumor-prone mice defective in both the Myh and Ogg1 DNA glycosylases. Cancer Res. 64, 4411–4414.
Schoeb, T.R., McConnell, E.E., Juliana, M.M., Davis, J.K., Davidson, M.K., Lindsey, J.R., 2009. Mycoplasma pulmonis and lymphoma in bioassays in rats. Vet. Pathol. 46, 952–959.
Skrzydlewska, E., Elas, M., Farbiszewski, R., Roszkowska, A., 2000. Effect of methanol intoxication on free-radical induced protein oxidation. J. Appl. Toxicol. 20, 239–243.
Soffritti, M., Belpoggi, F., Cevolani, D., Guarino, M., Padovani, M., Maltoni, C., 2002. Results of long-term experimental studies on the carcinogenicity of methyl alcohol and ethyl alcohol in rats. Ann. NY Acad. Sci. 982, 46–69.
Sweeting, J.N., Siu, M., McCallum, G.P., Miller, L., Wells, P.G., 2010. Species differences in methanol and formic acid pharmacokinetics in mice, rabbits and primates. Toxicol. Appl. Pharmacol. 247, 28–35.
Tephly, T.R., 1991. The toxicity of methanol. Life Sci. 48, 1031–1041.
Thurman, R.G., Handler, J.A., 1989. New perspectives in catalase-dependent ethanol metabolism. Drug Metab. Rev. 20, 679–688.
Tice, R.R., Agurell, E., Anderson, D., Burlinson, B., Hartmann, A., Kobayashi, H., Miyamae, Y., Rojas, E., Ryu, J.C., Sasaki, Y.F., 2000. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ. Mol. Mutagen. 35, 206–221.
Wang, M., Cheng, G., Villalta, P., Kassie, F., Hecht, S., 2008. abstract. Proceedings of the 99th Annual Meeting of the American Association for Cancer Research; Apr 12–16; San Diego, CA. pp. Abstract nr 2250.
Williamson, K.S., Hensley, K., Pye, Q.N., Ferrell, S., Floyd, R.A., 2003. Bioassay of 2'-Deoxyguanosine/8-Hydroxy-2'-Deoxyguanosine by HPLC with Electrochemical/Photodiode Array Detection. In: Hensley, K., Floyd, R.A. (Eds.), Methods in Biological Oxidative Stress. Human Press Inc., Totowa, NJ, pp. 137–149.
Wong, A.W., McCallum, G.P., Jeng, W., Wells, P.G., 2008. Oxoguanine glycosylase 1 protects against methamphetamine-enhanced fetal brain oxidative DNA damage and neurodevelopmental deficits. J. Neurosci. 28, 9047–9054.
Xie, Y., Yang, H., Cunanan, C., Okamoto, K., Shibata, D., Pan, J., Barnes, D.E., Lindahl, T., McIlhatton, M., Fishel, R., Miller, J.H., 2004. Deficiencies in mouse Myh and Ogg1 result in tumor predisposition and G to T mutations in codon 12 of the K-ras oncogene in lung tumors. Cancer Res. 64, 3096–3102.